

DIFFERENTIATION OF PSEUDOTHALLIAL TRACHEARY ELEMENTS IN *NICOTIANA TABACUM* TISSUE CULTURED *IN VITRO**

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ABSTRACT

Under conditions of high auxin (4,0 mg/l indoleacetic acid) and low cytokinin (0,08 mg/l kinetin) tobacco stem explants grown *in vitro* on nutrient agar produce masses of aerial pseudothalli, some of the cells of which differentiate into tracheary elements. Secondary wall thickening in these differentiating cells may commence either simultaneously over the whole surface of the cell, progressively increasing in thickness, or with a localised deposition of wall material which then spreads in a circular or triangular manner, leading to a partial or total covering of the primary wall. The secondary thickening is mostly reticulate-pitted.

With extensive proliferation the pseudothalli intergrow and vascular islets become established from the peripheral cells of which roots may arise.

UITTREKSEL

DIFFERENSIASIE VAN PSEUDOTALLI TRACHEËDELEMENTE IN *NICOTIANA TABACUM* WEEFSEL WAT *IN VITRO* GEKWEK IS.

Tabakstingel-eksplante wat *in vitro* op voedingsagar in die teenwoordigheid van hoë auksien (4,0 mg/l indoolasynsuur) en lae sitokinien (0,08 mg/l kinetien) gekweek word, produseer 'n massa pseudotalli, sommige van die selle waarvan differensieer om tracheëdelemente te vorm. Sekondêre wandverdikking in dié differensiërende selle begin of gelyktydig oor die hele selwand en verdik geleidelik of met 'n gelokaliseerde neerslag van wandmateriaal wat dan op sirkulêre of driehoekige wyse versprei om uiteindelik die primêre wand gedeeltelik of geheel te oordek. Die sekondêre verdikking is meestal getetikuleerd gestippeld.

Met ekstensiewe vermeerdering groei die pseudotalli in mekaar en word vaatweefsel-nodules gevorm van die buitenste selle waarvan wortelprimordia ontstaan.

INTRODUCTION

Up to now, comparatively little attention has been paid to what appears to be the common occurrence of isolated tracheary cells in the superficial zones of callus on the aerial side of tobacco tissue grown *in vitro*.

Gautheret (1957) pointed out that tracheary differentiation could take place in an isolated cell. This almost casual reference is significant because it appears that it is with these single, isolated tracheids that the histogenesis of vascular nodules and strands—and thus possibly also roots—have their origin.

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Steward, Mapes and Mears (1958) observed single tracheids but only in the central regions of their cultured material. However, we have consistently found tracheary cells differentiating in aerial pseudothalli, often even at the distal-most ends of these chains of connected cells growing into the air space. With repeated cell division and surface growth of the culture, the tracheary elements often become surrounded by parenchyma and eventually become organised into tracheary islets.

This study reports some of our observations on the origin and development of the single, pseudothallial-borne tracheary elements.

MATERIALS AND METHODS

The nutrient media, growth conditions, and preparation and culture of the tobacco (*Nicotiana tabacum*) tissue were reported in an earlier paper (Ellis and Bornman, 1970). Microscopic observations were made on fresh and macerated tissue as well as on scrapings of the callus surface, stained either with aniline blue or toluidine blue or safranin, or viewed under phase contrast.

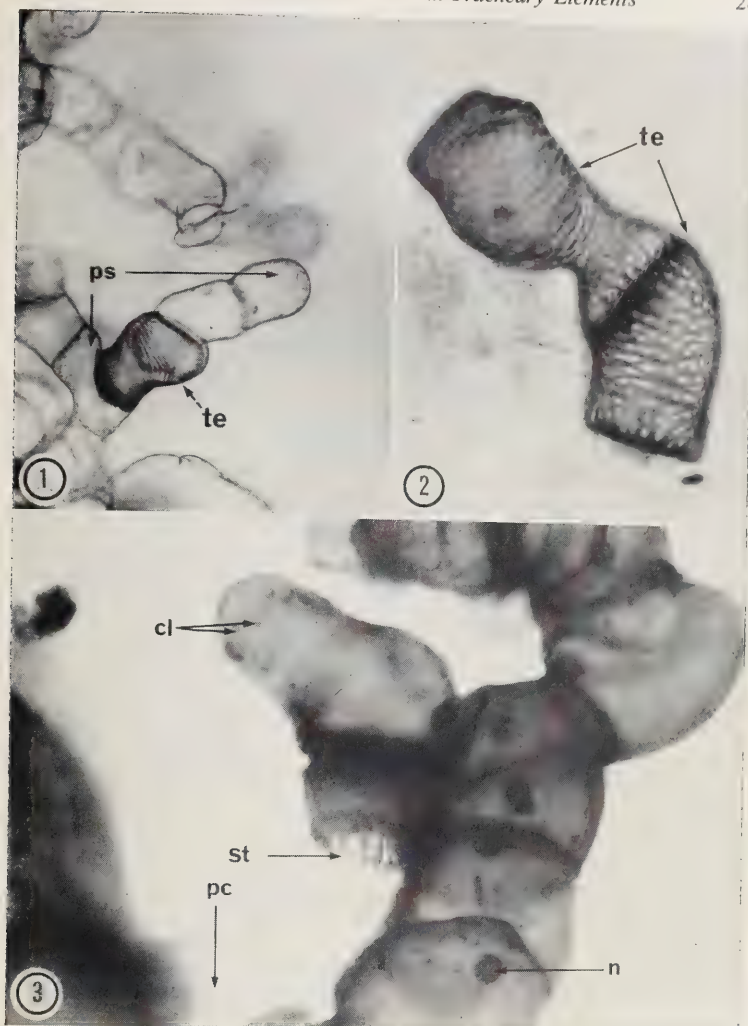
Particular attention was focused on tobacco callus cultured in the presence of 4,0 mg/l (high) indoleacetic acid and 0,08 mg/l (low kinetin).

RESULTS AND DISCUSSION

Auxin and cytokinin, when incorporated in the culture medium in the ratio of 4,0 mg/l indoleacetic acid to 0,08 mg/l kinetin, produce a frosty growth of tissue, dense, compact and green at its base but loose and white at its acropetal surface. Closer examination shows that this pustular growth consists of innumerable pseudothalli made up of multi-branched chains of up to 20 or more cells.

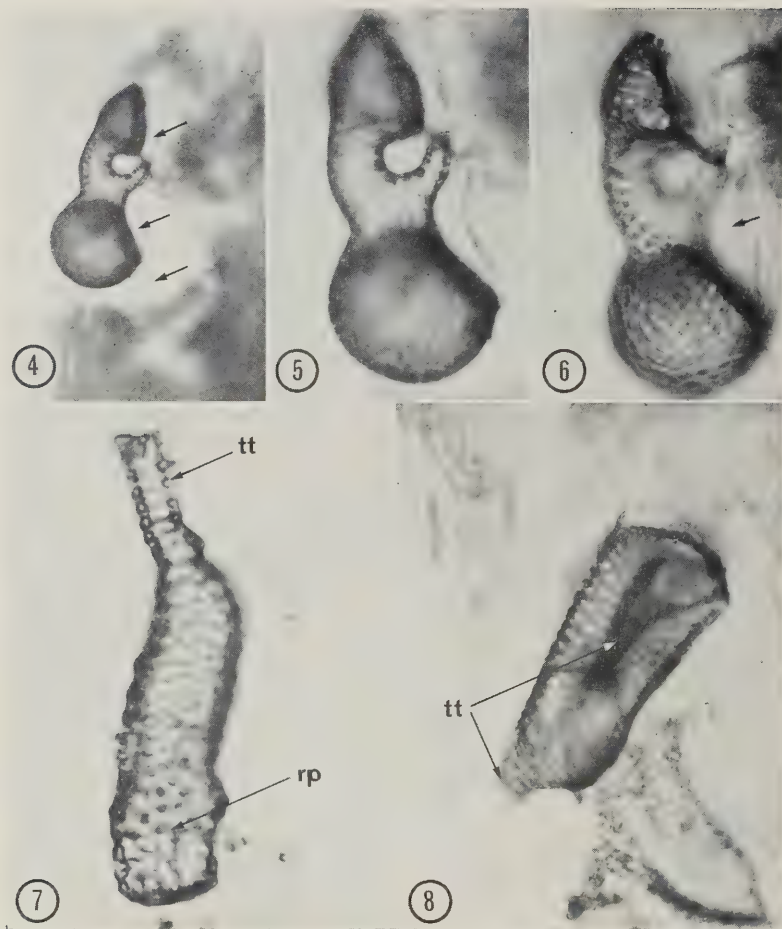
Tracheary elements. Single, isolated tracheary elements are most commonly found near the bases of pseudothalli (Fig. 1) but also anywhere along the pseudothallus, particularly at branching points. Figure 2 shows two tracheary cells, the focal planes of which clearly indicate the zig-zag nature of a developing pseudothallial chain. In Fig. 3 vertical branching has occurred as a result of a number of cellular divisions, and in one of these cells the localised development of secondary wall thickening at a very early stage is apparent. Pseudothalli are frequently branched, producing Y-shaped, T-shaped or multiforked structures. Invariably, the parenchyma cells located at such a junction undergo secondary wall thickening and become tracheid-like in appearance. Figures 4-6 are of such an irregularly-shaped tracheary cell at different levels of focus and magnification, showing the areas of attachment to various adjoining parenchyma cells.

Large parenchyma cells, approximately $150 \times 75 \mu\text{m}$, differentiating into tracheary elements, often develop a characteristic tail-like structure (Fig. 7) which fits snugly into a corresponding depression in an adjoining tracheary element (Fig. 8).



FIGS. 1-3.

Tracheary elements from fresh and macerated tissue. Fig. 1. Single, isolated tracheary cell near the base of a branched pseudothallus, safranin, $\times 160$. Fig. 2. Lignified, reticulate-pitted tracheary cells removed from macerated zig-zag chain of pseudothallial cells, toluidine blue, $\times 400$. Fig. 3. Secondary wall thickening commencing in a parenchyma cell of pseudothallus, toluidine blue, $\times 400$.
 cl, chloroplast; n, nucleus; pc, parenchyma cell; ps, pseudothallus; st, secondary thickening; te, tracheary element.

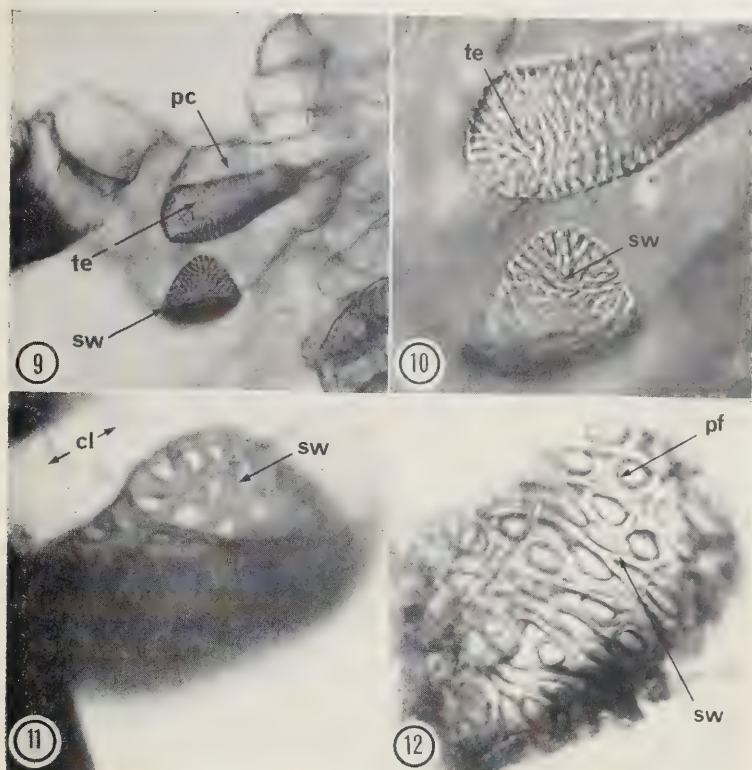


FIGS. 4-8.

Macerated pseudothalli. Fig. 4. Unusually shaped tracheary cell, toluidine blue, $\times 160$. Figs. 5-6. Higher magnifications of same showing surfaces where parenchyma cells were attached (arrows), $\times 400$. Fig. 7. Reticulately-pitted tracheid with conspicuous tail, safranin, $\times 640$. Fig. 8. Tracheary element with surface depression corresponding to fit tail from adjoining tracheary cell, safranin, $\times 400$.

rp, reticulate-pitted; tt, tracheary tail

Deposition of secondary wall material. The secondary wall thickenings of these primary tracheary elements appear to be laid down in one of two ways. Firstly, and most interesting, a small area of primary wall becomes covered



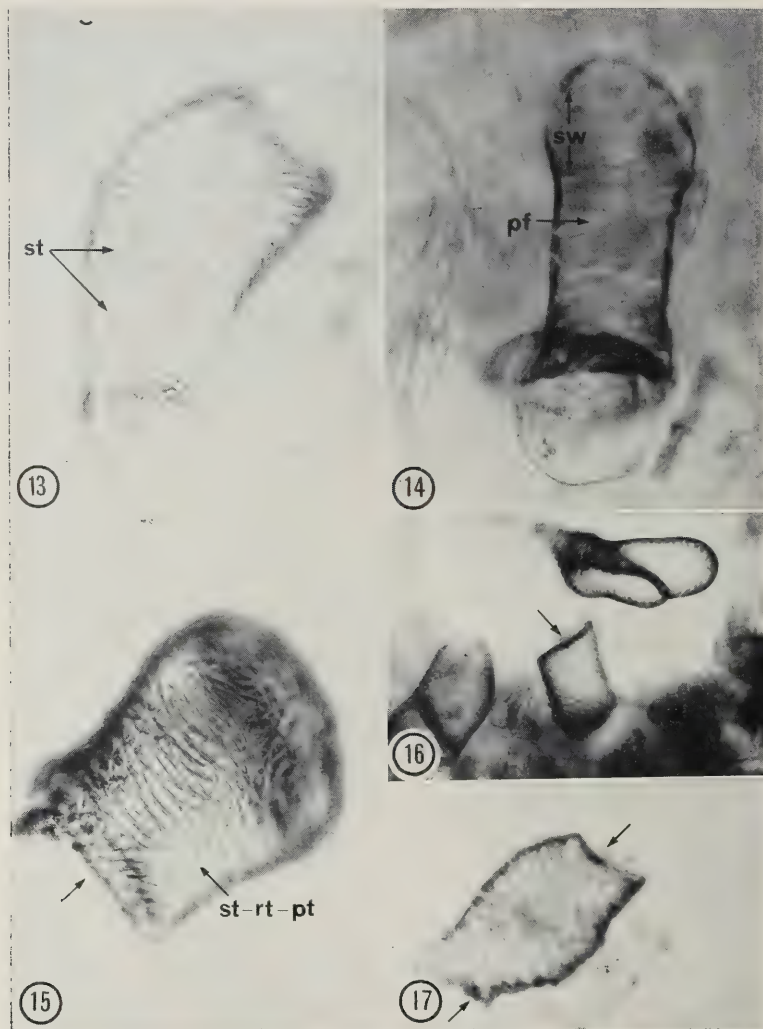
FIGS. 9-12.

Fresh pseudothallial tissue. Fig. 9. Partial secondary thickening of walls of two adjacent tracheary cells, aniline blue, $\times 160$. Fig. 10. Higher magnification of same showing nature of thickening which is reticulate-pitted, $\times 400$. Fig. 11. Pitted secondary thickening of wall of cell still retaining chloroplasts, toluidine blue, $\times 640$. Fig. 12. Outer surface of air-filled tracheid (resulting in clear definition) showing pitfields of primary wall overlain with strands of secondary thickening—evidence of exterior deposition of wall materials, safranin, $\times 1600$.

cl. chloroplast; pc. parenchyma cell; pf, primary pit field;

sw, secondary wall; te, tracheary element

with secondary wall material (Figs. 9-12) from where the latter subsequently spreads in circular or triangular manner until the primary wall is wholly or partially covered. Where secondary thickening remains localised to only a part of the wall such cells appear to remain physiologically active. Secondly, a simultaneous and progressive deposition of secondary wall material leads to a thickening of the wall of the whole cell (Figs. 13-17).



Figs. 13-17.

Macerated pseudothalli. Fig. 13. Scalariform to reticulate secondary wall thickening in tracheary cell situated between two parenchyma cells of pseudothallus, safranin, $\times 400$. Fig. 14. Differentiating tracheary element in which secondary wall is laid down simultaneously in all parts of cell. Pit fields in primary wall are not covered, toluidine blue, $\times 400$. Fig. 15. Air-filled tracheary cell from terminal position on pseudothallus with vessel-like endplate (arrow). Thickening varies from scalariform to reticulate to pitted, safranin, $\times 400$. Figs. 16-17. Isolated tracheary cells. $\times 120$ and $\times 140$, respectively. Note vessel-like endplates (arrows), toluidine blue.

pf, pit field; st-rt-pt, scalariform to reticulate to pitted thickening; sw, secondary wall

Extraordinarily, in some cells secondary wall thickening clearly appears to occur on the outer surface of the cell wall (Fig. 12, 15). A definitive ultrastructural study would be necessary to elucidate the sequence of events involved here.

As regards type, there appears to be an intergradation of thickening from scalariform to reticulate to pitted, with the majority of elements probably best described as reticulate-pitted.

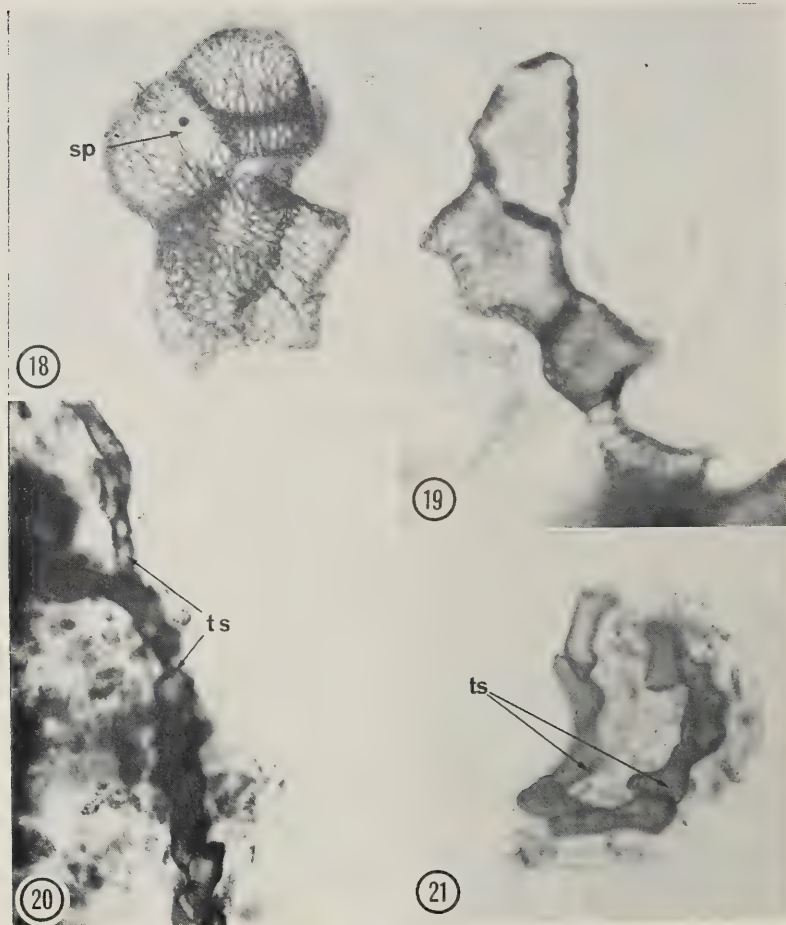
Function. It must be borne in mind that secondary wall formation in the differentiating tracheary elements of the pseudothallus is not influenced by surrounding cells; usually there are no adjoining cells. In partially differentiated tracheary cells functional chloroplasts are present (Fig. 11) but fully differentiated elements are devoid of contents. Parenchyma cells situated above fully differentiated tracheary elements eventually senesce and die or else differentiate, fully or partially, into tracheid-like cells. Consequently, it is unlikely that the function of these tracheary elements is one of conduction or storage; it probably is simply mechanical. Therefore, whether to refer to these tracheary elements as tracheids or vessels becomes a tenuous question.

Vessel-like members with what appear to resemble simple perforation plates (Fig. 16, 17) are found occasionally, as are tracheary cells with large, simple perforations scattered over the wall surface (Fig. 18). The usual ontogenetic progression of secondary wall thickenings apparent in the tracheary cells of primary xylem (Esau, 1960), is not a feature of the pseudothallial tracheary elements.

Aggregations of tracheary elements. Aggregations of tracheary elements in the compact, newly-proliferated tissue of the explant, appear to develop in one of three ways. Pseudothalli tend to intergrow and, particularly at the explant surface, become surrounded by parenchyma. Firstly, a localised differentiation of a few cells into tracheary elements occurs (Fig. 18). Surrounding such an islet of xylary cells and probably limiting its access to the external environment is a cambium-like sheath. Further development results in the formation of vessels and there is at least some evidence that roots arise from the peripheral cells of these vascular islets or nodules. Secondly, secondary wall thickening and lignification may occur in adjoining cells in one plane, probably along a hormonal and/or nutrient gradient, resulting in columnar files of tracheary elements (Fig. 19, 20). Thirdly, tracheary elements form in circular groups enclosing parenchyma in the centre (Fig. 21).

CONCLUSION

It is difficult to follow the development of the vascular tissues from serial sections because of the haphazard courses adopted by the xylary formations described above. Tracheary element and vascular nodule formation definitely



FIGS. 18-21.

Macerated tissue from dense, intergrown pseudothallial mass. Fig. 18. Islet of tracheary elements from within cambium-like zone. Note simple perforations which probably serve as vessel endplates, safranin, $\times 400$. Figs. 19-20. Columnar vascular strands, toluidine blue, $\times 400$ and $\times 150$, respectively. Fig. 21. Circular strand of tracheary elements enclosing parenchyma, toluidine blue, $\times 150$.

sp, simple perforation; ts, tracheid strand

are stimulated by high levels of auxin, and it seems clear that isolated pseudothallial tracheary cells, with subsequent parenchymatous proliferation, ultimately give rise to aggregations of vascular tissue from the surrounding cambial-like

cells of which roots have their origin. The function, as well as the factors which control differentiation of tracheary cells in a pseudothallus, remains obscure.

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